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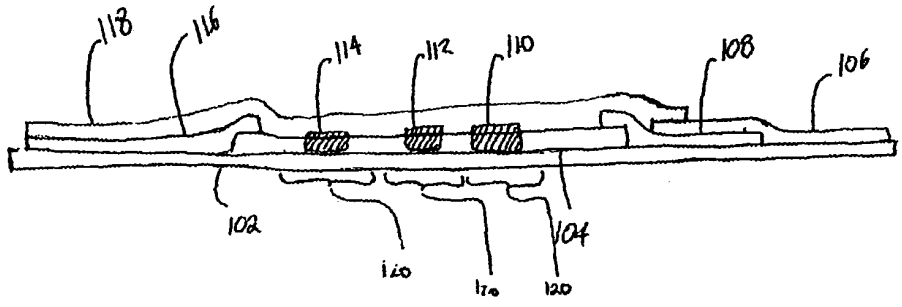

(57) Abstract

Disclosed are lateral flow assays (test strips) characterized by: a) one or more control zones having a control agent (e.g. dinitrophenol) immobilized thereto; b) detection agent consisting of a conjugate of analyte–binding agent (e.g. Helicobacter pylori extract or HIV envelope antigen), control–binding agent (e.g. anti–dinitrophenol antibody) and label (e.g. 16 nm colloidal gold, enabling reflectance measurements).

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Improved Lateral Flow Assays

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to lateral flow assays, more particularly, the invention relates to improved lateral flows assays wherein an analyte binding agent is coupled to a detection agent and an analyte non-specific agent.

Description of Related Art

Quantitative analysis of cells and analytes in fluid samples, particularly bodily fluid samples, often provides critical diagnostic and treatment information for physicians and patients. For example, immunological testing methods, which take advantage of the high specificity of the antigen-antibody reaction, provide one approach to measurement of analytes. Kennedy, D. M. and S. J. Challacombe, eds., ELISA and Other Solid Phase Immunoassays: Theoretical and Practical Aspects, John Wiley and Sons, Chichester (1988). This document and all others cited to herein, are incorporated by reference as if reproduced fully below. Such assays may also find use in various other applications, such as veterinary, food testing, or agricultural applications.

Immunoassays that provide a quantitative measurement of the amount of an analyte in a sample have previously used complex, multi-step procedures and expensive analyzers available only in a laboratory setting.

Immunochromatographic assays, such as those described in GB 2,204,398A; U.S. Pat. Nos. 5,096,837, 5,238,652, and 5,266,497; Birnbaum, S. et al., Analytical Biochem. 206:168-171 (1992); Roberts, M. A. and R. A. Durst, Analytical Chem. 67:482-491 (1995); and Klimov, A. D. et al., Clinical Chem. 41:1360 (1995), are simpler, yet do not provide a quantitative measurement of an analyte. Instead, these immunochromatographic assays

detect the presence (or absence) of an analyte above a defined cutoff level for the test performed. The lack of a quantitative measurement limits the usefulness of these assays.

Cathey, et al, U. S. Patent Number 5,660,993, discloses a disposable diagnostic assay device and method for its use. The device comprises a sample addition port in fluid communication with at least one main channel. The main channel comprises, in a direction of fluid flow, a main reagent area in fluid communication with an incubation area and a waste area. In fluid communication with the main channel is at least one side reagent channel. The side reagent channels comprise, in a direction of fluid flow, a liquid addition port and a side reagent area in fluid communication with the main channel at a region of the main channel upstream from the incubation area. Agitation means may be included in at least one of the main and side reagent areas and/or the incubation area. Capillary valves may be located at various positions along the main and side reagent channels upstream from the incubation area, providing for control over fluid flow through the device. The complicated mechanical nature of this device renders it unsuitable for repeated, accurate, use.

Palace, et al, International Publication Number WO 92/12428, discloses a design for a nonbibulous lateral flow one-step assay for an analyte in biological sample using a lateral flow strip. In the disclosed device, three zones that are in nonbibulous lateral flow contact are employed: a sample receiving zone, a labeling zone, and a capture zone. The sample containing analyte is carried through the labeling zone and interacts with an assay label comprising a visible moiety, preferably particles, which are coupled to a specific binding reagent for an analyte or to a competitor with analyte for a capture reagent. The flow continues into the capture zone with some visible moieties to which analyte or competitor are coupled or captured. Excess fluid is absorbed into an absorbent zone in contact with the capture zone. A positive result is obtained by visualizing the visible moieties in the capture zone. Control label comprising visible moieties (preferably, visually distinguishable from those of the assay

label) may also be included in the labeling zone and captured in a separate control portion of the capture zone to verify that the flow of liquid is as expected. However, the control label does not correct for the variability between lateral flow strips, leading to inaccurate and inconsistent measurements. Nor does proof of movement of the control label across the control portion of the capture zone provide proof of movement of the assay label moiety across the analyte specific portion of the capture zone.

Eisinger, et al, U. S. Patent Number 4,943,522, discloses a method and apparatus for conducting specific binding pair assays, such as immunoassays. A porous membrane capable of non-bibulous lateral flow is used as an assay substrate; a member of the binding pair is affixed in an indicator zone defined in the substrate. The sample is applied at a position distant from the indicator zone and permitted to flow laterally through the zone; any analyte in the sample is completed by the affixed specific binding member, and detected. However, this assay does not have any controls, which can lead to testing inaccuracies and errors.

Campbell, et al, U. S. Patent Number 4,703,017, discloses a solid-phase assay for an analyte wherein binder is supported on a solid support, such as nitrocellulose, and the tracer comprises ligand labeled with a colored particulate label, such as a liposome, including a dye. The assay has high sensitivity, and the tracer is visible on the support under assay conditions, whereby tracers is disclosed as determined, without instrumentation, and without further treatment thereof. However, because of the lack of instrumentation, human subjectivity is introduced into the assay, leading to inaccuracy and error.

Campbell, et al, U. S. Patent Number 4,743,560, discloses a solid-phase assay for an analyte wherein binders are supported on a solid support, such as nitrocellulose, and the tracer is comprised of ligand labeled with a particulate label, such as a liposome, including a detectable marker which is not visible. However, this assay does not have any controls, which can lead to testing inaccuracies and errors.

Brooks, U.S. Patent No. 5,753,517, discloses methods of measuring the amount of an analyte of interest in a fluid sample, using a quantitative immunochromatographic assay, and an apparatus for use in the assay. The assay utilizes a rapid antigen measurement platform (RAMP®) apparatus. The apparatus includes a membrane strip made of a material such as cellulose nitrate or glass fiber that has sufficient porosity and the ability to be wet by the fluid containing the analyte, and allows movement of particles by capillary action.

The membrane strip is disclosed as having an application point, a contact region, and a detection zone; the contact region is between the application point and the detection zone. Imbedded in the contact region is a population of particles, such as colloidal metal particles, organic molecules, liposomes, or organic polymer latex particles. The particles are coated with an antibody to the analyte of interest. The particles can be labeled, using a colorimetric, fluorescent, luminescent, or other appropriate label, to facilitate detection. A detection reagent is immobilized in the detection zone. The detection reagent can be an antibody to the analyte of interest, or can be the analyte of interest itself. The apparatus can also include one or more of the following features: an application pad that rests on and covers the application point; a contact pad that rests on and covers the contact region, and which may have antibody-coated particles imbedded within it. If a contact pad is present, a separator pad is disclosed as present and resting on the membrane in between the contact region and the contact pad. Also disclosed are a wicking pad that may rest on the membrane adjacent to the detection zone, such that the detection zone is between the wicking pad and the contact region; and an internal control that includes internal control particles imbedded in the contact region, a control detection reagent, and a control reaction zone.

The apparatus includes an internal control for the disclosed purpose of compensating for variations in membrane properties from assay to assay. The internal control includes internal control particles, a control detection reagent,

and a control reaction zone. Internal control particles are disclosed as imbedded in the contact region with the antibody-coated particles.

The "internal control particles" are similar to the antibody-coated particles. They are disclosed as coated with the same surface concentration of an antibody, except that the antibody on the internal control particles is directed against a control detection reagent that does not react with the antibody directed against the analyte. The "control detection reagent" is disclosed as a reagent that does not interact with either the analyte to be measured, the antibody on the antibody-coated particles, or the detection reagent. The control detection reagent is coated on the membrane on the "control reaction zone". The control reaction zone, as disclosed, refers to a point on the membrane strip at which the control detection reagent is immobilized. However, because of the separate nature of the control and analyte detection reagents, the movement of the control through the lateral flow matrix cannot be proven to mimic the movement of the analyte detection reagents. Such control agents therefore function poorly in methods designed to compensate for assay variability by measuring the relative response to control and analyte detection agents in each lateral flow device.

There is therefore a need for lateral flow immunoassays and methods of using them that address the problems noted above.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to a method of performing a lateral flow assay comprising contacting an analyte of interest with a first analyte binding agent, wherein the first analyte binding agent and an analyte non-specific agent are coupled to a detection agent.

In another aspect, the invention relates to a method, in a lateral flow assay that comprises a test strip, of increasing the difference between an average positive result of the assay and an average negative result of the assay

comprising spatially relocating on the test strip one or more control binding zones with respect to an analyte binding zone.

In still another aspect, the invention relates to a method, in a lateral flow assay that comprises a test strip, of reducing the coefficient of variability of the lateral flow assay comprising spatially relocating on the test strip one or more control binding zones with respect to an analyte binding zone.

In a further aspect, the invention relates to a method, in a lateral flow assay, of increasing the reproducibility of the lateral flow assay comprising quantifying a first amount of detection agent present in a first control zone; quantifying a second amount of detection agent present in a second control zone; mapping the quantified first and second amounts of detection agent onto a relative scale to arrive at relative first and second amounts of detection agent; and wherein the mapping is performed such that the ratio of the quantified first amount of detection agent to the quantified second amount of detection agent is greater than the ratio of the relative first amount of detection agent to the relative second amount of detection agent.

In an aspect, the invention relates to a lateral flow assay comprising a test strip comprising an analyte binding agent and an analyte non-specific agent coupled to a detection agent.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a cross-section of an embodiment of a lateral flow test strip according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

The term, "analyte," as used herein, refers to the molecule or compound to be quantitatively determined. Examples of analytes include proteins, such as hormones and other secreted proteins, enzymes, and cell surface proteins;

glycoproteins; peptides; small molecules; polysaccharides; antibodies (including monoclonal or polyclonal Ab and portions thereof); nucleic acids; drugs; toxins; viruses or virus particles; portions of a cell wall; and other compounds possessing epitopes. The analyte of interest preferably comprises an immunogenic portion, meaning that antibodies (as described below) can be raised to that portion of the analyte of interest.

To conduct the lateral flow assays of the current invention, a lateral flow test strip may be used. FIG. 1 depicts an embodiment of the test strip according to the invention. Test strip 100 includes backing strip 102, membrane strip 104, separation pad 106, conjugate pad 108, test zone 110, low control zone 112, high control zone 114, absorbent pad 116, and protective covering 118. Test strip 100 comprises a distal and proximal end. Examples of suitable test strip arrangements include those available from Scripps Laboratories as the Scripps Laboratories Lateral-Flow Immunoassay Development System.

Mounted centrally on backing strip 102 is membrane strip 104. Located distally from membrane strip 104, with a slight overlap with membrane strip 104, is conjugate pad 108. Located distally on backing strip 102 from conjugate pad 108, in a nearly fully overlapping manner, is separation pad 106. Additionally located on backing strip 102 is absorbent pad 116, which is in contact with and proximal from membrane strip 104. Protective covering 118 is coupled to separation pad 106, membrane strip 104, and absorbent pad 116 in an opposing relationship to backing strip 102.

Backing strip 102 may be made of a stable, non-porous material that is sufficiently strong to support the materials and strips coupled to it. Generally, backing strip 102 is substantially impervious to water. In a preferred embodiment, backing strip 102 is made of a polymer film, more preferably a poly(vinyl chloride) film.

Test strip 100 also comprises absorbent pad 116. The absorbent pad comprises an absorbent substance that may soak up solution that has been transported by capillary action to the end of membrane strip 104. Examples of

substances suitable for use in absorbent pad 116 include cellulose and glass fiber.

Protective covering 118 is coupled to membrane strip 104 in an opposing relationship to backing strip 102. Protective covering 118 protects or covers membrane strip 104, conjugate pad 108, absorbent pad 116 and optionally separation pad 106 Protective covering 118 may be made of a material generally impervious to water, and is preferably translucent or transparent. Protective covering 118 may be a single or multiple layers. Preferable materials for use in protective covering 118 comprise optically transmissive materials such as polyamide, polyester, polyethylene, acrylic, glass, or similar materials. The protective covering 118 may be clear or not clear depending on method of detection used. In a preferable embodiment, protective covering 118 is optically clear polyester.

Membrane strip 104 may be made of a substance having the following characteristics: sufficient porosity to allow capillary action of fluid along its surface and through its interior; the ability to allow movement of antibody- or antigen-coated particles by capillary action (i.e., it preferably does not block the particles); and the ability to be wet by the fluid containing the analyte (e.g., hydrophilicity for aqueous fluids, hydrophobicity for organic solvents). Hydrophobicity of a membrane can be altered to render the membrane hydrophilic for use with aqueous fluid, by processes such as those described in U.S. Pat. No. 4,340,482, or U.S. Pat. No. 4,618,533, which describe transformation of a hydrophobic surface into a hydrophilic surface. Examples of membrane substances include: cellulose, nitrocellulose, cellulose acetate, glass fiber, nylon, polyelectrolyte ion exchange membrane, acrylic copolymer/nylon, and polyethersulfone. In a preferred embodiment, the membrane strip is made of nitrocellulose.

Separation pad 106 may be made of an absorbent substance that can deliver a fluid sample, when applied to separation pad 106, to conjugate pad 108. Preferred substances include, but are not limited to, cellulose,

nitrocellulose, cellulose acetate, glass fiber, nylon, polyelectrolyte ion exchange membrane, acrylic copolymer/nylon, and polyethersulfone.

Conjugate pad 108 is coupled to separation pad 106. Conjugate pad 108 may be made of an absorbent substance; representative substances include cellulose, cellulose nitrate, cellulose acetate, glass fiber, nylon, polyelectrolyte ion exchange membrane, acrylic copolymer/nylon, and polyethersulfone.

The conjugate pad may contain a population of first analyte binding agent and analyte non-specific agent coupled to a detection agent. In alternative embodiments, this population may be present elsewhere in the test strip, either exclusively or non-exclusively. Analyte non-specific agent is defined as an agent non-specific to the analyte of interest. The first analyte binding agents are agents that specifically bind to the analyte of interest. More than one population of first analyte binding agent and analyte non-specific agent coupled to a detection agent may be present in the test strip. These populations may be the same or different in composition, location or other characteristics.

In a preferable embodiment, the first analyte binding agents are antibodies to the analyte of interest. In another preferable embodiment, if the analyte of interest is an antibody of known specificity, the population may comprise the antigen against which the analyte-antibody is directed. The antibodies can be monoclonal antibodies or polyclonal antibodies. The term "antibody", as used herein, also refers to antibody fragments that are sufficient to bind to the analyte of interest.

Alternatively, in a preferable embodiment, molecules that specifically bind to the analyte of interest, such as engineered proteins, peptides, haptens, and lysates containing heterogeneous mixture of antigens having analyte binding sites, may also be used. P. Holliger et al., Trends in Biotechnology **13**:7-9 (1995); S. M. Chamow et al., Trends in Biotechnology **14**:52-60 (1996). In another embodiment, if the analyte of interest is a ligand, a receptor which binds to the ligand can be used, and vice versa.

The first analyte binding agent and analyte non-specific agent are coupled to a detection agent. The detection agent may comprise a wide variety of materials, so long as it facilitates detection of the first analyte binding agent to which the detection agent is coupled. Suitable detection agents comprise particles, luminescent labels; colorimetric labels, fluorescent labels; chemical labels; enzymes; radioactive labels; or radio frequency labels; metal colloids; and chemiluminescent labels.

In a preferable embodiment, at least one first analyte agent and at least one analyte nonspecific agent are coupled to a single detection agent. Such an embodiment comprises a single population of detection agent moieties coupled to at least one first analyte agent and at least one analyte nonspecific agent. This embodiment, as discussed further below, may serve to reduce non-specific binding and may correct for certain types of assay variability.

Different detection agents may be used if different populations of first analyte binding agent and analyte non-specific agent coupled to a detection agent are used. This situation may arise, for example, when it is desired to assay two different analytes of interest on the same test strip. Use of two different detection agents facilitates detection of the two different analytes of interest. For example, when the detection agent is a fluorescent agent, the detection agents may be selected to fluoresce at different wavelengths.

In a preferable embodiment, the detection agent is a particle. Examples of particles useful in the practice of the invention include, but are not limited to, colloidal gold particles; colloidal sulphur particles; colloidal selenium particles; colloidal barium sulfate particles; colloidal iron sulfate particles; metal iodate particles; silver halide particles; silica particles; colloidal metal (hydrous) oxide particles; colloidal metal sulfide particles; colloidal lead selenide particles; colloidal cadmium selenide particles; colloidal metal phosphate particles; colloidal metal ferrite particles; any of the above-mentioned colloidal particles coated with organic or inorganic layers; protein or peptide molecules; liposomes; or organic polymer latex particles, such as polystyrene latex beads.

Preferable particles are colloidal gold particles. The size of the particles may be related to porosity of the membrane strip: the particles are preferably sufficiently small to be transported along the membrane by capillary action of fluid.

Colloidal gold may be made by any conventional means, such as the methods outlined in G. Frens, 1973 Nature Physical Science, **241**:20 (1973). Alternative methods may be described in U.S. Patent Nos. 5,578,577, 5,141,850; 4,775,636; 4,853,335; 4,859,612; 5,079,172; 5,202,267; 5,514,602; 5,616,467; 5,681,775.

The selection of particle size may influence such factors as stability of bulk sol reagent and its conjugates, efficiency and completeness of release of particles from conjugate pad 108 , speed and completeness of the reaction. Also, particle surface area may influence stearic hindrance between bound moieties.

The particles may be labeled to facilitate detection. Examples of labels include, but are not limited to, luminescent labels; colorimetric labels, such as dyes; fluorescent labels; or chemical labels, such as electroactive agents (e.g., ferrocyanide); enzymes; radioactive labels; or radio frequency labels.

The number of particles present in the test strip may vary, depending on the size and composition of the particles, the composition of the test strip and membrane strip , and the level of sensitivity of the assay. The number of particles typically ranges between about $1X10^9$ and about $1X10^{13}$ particles, although fewer than about $1X10^9$ particles may be used. In a preferred embodiment, the number of particles is about $1X10^{11}$ particles.

Also coupled to the detection agent is a analyte non-specific agent. This analyte non-specific agent may be selected for its ability to bind to substances other than the analyte of interest. For example, if the analyte of interest is an antibody to *H. Pylori*, then the analyte non-specific agent may be an antibody to an antigen not found, or rarely found, in the antibody to *H. Pylori*. This binding

may be specific for a substance other than the analyte of interest or non-specific for such a substance.

In a preferable embodiment, the analyte non-specific agent may be antibodies, more preferably rabbit IgG. The antibodies can be monoclonal antibodies or polyclonal antibodies. The term "antibody", as used herein, also refers to antibody fragments that are sufficient to bind to the analyte of interest. Alternatively, preferably, molecules such as engineered proteins having binding sites non-specific for the analyte of interest, may also be used. In another embodiment, a receptor that specifically binds to ligands other than the analyte of interest can be used, and vice versa. Additionally, the analyte non-specific agent may be an antigen, another organic molecule, or a hapten conjugated to a protein non-specific for the analyte of interest. Descriptions of other suitable analyte non-specific agents may be found in U.S. Patent No. 5,096,837, and include lgG, other immunoglobulins, bovine serum albumin (BSA), other albumins, casein, and globulin.

In a preferable embodiment, the analyte non-specific agent comprises a control binding agent. Control binding agents are selected so as to bind specifically to molecules other than molecules that specifically bind to the analyte of interest. In this way, these control binding agents can bind to control zones, as discussed below. Substances useful as control binding agents include those substances described above as useful as first analyte binding agents. In a preferable embodiment, the control binding agent comprises rabbit anti-dinitrophenol (anti-DNP) antibody. Additional beneficial characteristics of control binding agents include, but are not limited to stability in bulk, non-specificity for analyte of interest, reproducibility and predictability of performance in test, molecular size, and avidity of binding to the control agent.

The coupling of the first analyte binding agent and the analyte non-specific agent to the detection agent may be covalent or non-covalent (e.g., ionic, hydrogen bonding, Van der Waals forces, etc.). Conventional coupling chemistries and techniques are suitable for use in this invention. Further, each

of the first analyte binding agent, the detection agent and the analyte non-specific agent may be coupled to one another, through one another, or through linker, carrier or spacer groups.

For example, if particles are used as the detection agent, both first analyte binding agent and analyte non-specific agent may be coupled by non-specific adsorption to the detection agent. Alternatively, traditional conjugate chemistry may be used to covalently couple both the first analyte binding agent and the analyte non-specific agent to the particle. Alternatively, a non-covalent binding system, such as biotin-avidin, or even a second antibody specific for the first analyte binding agent to be detected, may be used to couple a detection agent such as a fluorescent label to the first analyte binding agent, and the analyte non-specific agent may be directly coupled to the first analyte binding agent. In another alternative embodiment, bifunctional or multifunctional reagents may be used to couple the first analyte binding agent, the detection agent and the analyte non-specific agent. The number of first analyte binding agents and analyte non-specific agents coupled to the detection agent may vary as appropriate to the particular embodiment. For example, two first analyte binding agents may be coupled to one detection agent and one analyte non-specific agent. Alternatively, two analyte non-specific agents may be coupled to one detection agent and one first analyte binding agent. Other variations on these arrangements will no doubt occur to one of skill in the art, and as such are encompassed within the scope of the invention.

Located on test strip 100 are a number of detection zones 120. Each detection zone is located such that an automatic or semi-automatic analytical instrument, or a human reader, may determine certain results of the lateral flow assay. The detection zone comprises a measurement zone, in which measurements of certain results of the lateral flow assay may be performed. The detection zone may comprise one or more analyte binding zones and/or one or more control binding zones.

Analyte binding zones are areas of the membrane strip that comprise a second analyte binding agent. One or more of the substances discussed above as suitable first analyte binding agents may be used as second analyte binding agents in the analyte binding zone. In a preferable embodiment, the first analyte binding agent is an antigen recognized by the analyte of interest, which is an antibody. The second analyte binding agent may also be the antigen or even a second antibody specific for the analyte (antibody) of interest. In another preferable embodiment, the analyte of interest is an antigen. The second analyte binding agent, which is an antibody, may be directed against a different epitope of the analyte compared to the first analyte binding agent, when the latter is also an antibody. Alternatively, when the analyte is an antigen with multiple copies of the same epitope, the second analyte binding agent may be directed against the same epitope as the first analyte binding agent.

In certain embodiments, one or more measurement zones may also comprise at least one control binding zone. A control binding zone comprises at least one control agent. A control agent binds specifically to the control binding agent to form a control binding pair.

A particular advantage of the control binding pairs according to the invention is that they are internal controls -- that is, the control against which the analyte measurement results may be compared is present on the individual test strip. Therefore, the controls according to the invention may be used to correct for strip-to-strip variability. Such correction would be impractical with external controls that are based, for example, on a statistical sampling of strips. Additionally, lot-to-lot and run-to-run, variations between different test strips may be minimized by use of control binding agents and control agents according to the invention. Furthermore, the effects of non-specific binding, as discussed further below, may be reduced. All of these corrections would be difficult to accomplish using external, off-strip controls.

The inventive lateral flow device may possess more than one control binding zone on the test strip. In such embodiments, the control binding zones

may be made to create a calibration curve against which a wide variety of analyte measurement results may be compared. Thus, possessing more than one internal control may permit making of lateral flow assays possessing a wider dynamic range than conventional lateral flow assays. In preferable embodiments, multiple control binding zone test strips are used with a relative scale methodology, discussed further below, that permits mapping of amounts of control binding pairs detected onto the same scale on which amounts of analyte detected are reported.

In a preferable embodiment, inventive assays will have at least one high control binding zone and at least one low control binding zone. The difference between the two zones is generally one of concentration. The concentration of control agent in the high control binding zone is greater than the concentration of control agent in the low control binding zone. Thus, the amount of control binding pairs will be higher in the high control zone versus the low control zone. In embodiments where the amount of control binding pairs in a given control zone may be mapped onto the same measurement scale on which the amount of analyte is reported, a calibration curve may be drawn through the values of the binding pairs in the high and low control binding zones.

In other embodiments, more than two control binding zones may be present. This allows for a curve to be generated that better reflects any nonlinearities present in the assay between the amount of analyte detected and the measurement against which the amount might be mapped, as discussed below. While such nonlinearities might otherwise affect assays that assume a relatively linear relationship, they can be corrected for using multiple control binding zones.

In another embodiment, a single control binding zone may comprise more than one type of control agent. This may be of use in embodiments where there are more than one population of analyte binding agents and analyte non-specific agents coupled to a detection agent. For example, when it is desired to assay two or more analytes of interest on the same assay strip, two populations

of analyte binding agents and analyte non-specific agents coupled to a detection agent may be prepared. Different detection agents may be used for each population, allowing a distinction to be drawn between results for the two different analytes of interest. In such circumstances, it may be desirable to use control binding zones comprising different control agents or control binding pairs.

Substances useful as control agents comprise those substances listed above that are suitable for use as control binding agents, save that the control agents bind specifically to the control binding agents. For example, in a preferable embodiment, the control binding agent is rabbit anti-DNP IgG, and the control agent is DNP conjugated to BSA (bovine serum albumin).

The control binding zones may be located in a variety of locations within the measurement zone that comprises it, and measurement zones comprising control binding zones may located in a variety of locations on the test strip. Rearrangement of control binding zones relative to analyte binding zones and reservoir(s) of the population of first analyte binding agent, analyte non-specific agent, and detection agent may be desirable in differing assays. For example, when the reservoir containing the population is located in the conjugate pad, it may be desirable in certain embodiments to locate the analyte binding zone(s) between the conjugate pad and the control binding zone(s). In other embodiments, it may be desirable to locate the control binding zone(s) between the analyte binding zone(s) and the conjugate pad.

Further, the order of placement of the control binding zones and analyte binding zones on the strip relative to the direction of fluid flow may affect the absolute binding of the analyte binding agent to the detection zone and the control binding agent to the control detection zone. This effect may result in an increase or decrease of the ratio of the control to detection analyte measurements, especially in cases where very strong positive specimens are tested. Such changes may have an impact on assay cutoff, if one is used; on

assay precision at the cutoff; or on overall assay dynamic range and reproducibility.

In operation, performing the inventive lateral flow assay may begin by obtaining a fluid sample containing the analyte of interest. The fluid can be a fluid that wets membrane strip 104; that supports an antibody/antigen reaction (i.e., does not interfere with antibody/antigen interaction); and/or that has a viscosity that is sufficiently low to allow movement of the fluid by capillary action. In a preferable embodiment, the fluid is an aqueous solution (such as a bodily fluid).

In a first embodiment of the inventive assay, separation pad 106 is contacted with the fluid sample containing the analyte of interest. After separation pad 106 is contacted with the fluid sample containing the analyte of interest, test strip 100 is maintained under conditions that allow fluid to transport the analyte of interest by capillary action to conjugate pad 108.

When the analyte of interest is transported to conjugate pad 108, analyte of interest present in the fluid contacts the first analyte binding agent coupled to the detection agent, of which conjugate pad 108 is comprised. At this point, contact also occurs between substances contained in the fluid and the analyte non-specific agent coupled to the detection agent. Both specific binding and non-specific binding may occur as a result of these contacts, to form a variety of complexes. It is preferable that all or nearly all of the specific binding take place between the analyte of interest and the first analyte binding agent.

Capillary action of the fluid from the fluid sample mobilizes the first analyte binding agent (coupled to the detection agent and the analyte non-specific agent), bound or unbound, moving it laterally to membrane strip 104. Additionally, complexes comprising non-specifically bound detection agent might form and grow in size, particularly if the various agents involved bind non-specifically to other components present in the sample. Capillary action may also mobilize such complexes, even if they are very large.

During mobilization, protective covering 118 minimizes loss of fluid through evaporation and resulting evaporative cooling, resulting in improved consistency and reproducibility, although it is not required to practice the invention. Retaining proper fluid volume is important for completeness of fluid flow along test strip 100. Prevention of evaporative cooling is useful for speed and completeness of the assay reactions. Additionally, protective covering 118 functions to keep infectious agents self-contained within the test strip 100.

The movement of the first analyte binding agent, together with other complexes, may be arrested as it reaches a measurement zone within a detection zone. If the measurement zone includes second analyte binding agent specific for the analyte of interest or the control binding agent, then the first analyte binding agent and/or the control binding agent, and anything coupled or complexed to them, directly or indirectly, may be immobilized at the measurement zone.

Once the first analyte binding agent and/or the control binding agent, and anything coupled or complexed to them, directly or indirectly, are immobilized at the measurement zone, their presence may be detected using the detection agent. The amount of detection agent arrested in the measurement zone may be quantified using conventional techniques. For example, optical methods, such as measuring light scattering, simple reflectance, luminometer or photomultiplier tube; radioactivity (measured with a Geiger counter, etc.); electrical conductivity or dielectric (capacitance); electrochemical detection of released electroactive agents, such as indium, bismuth, gallium or tellurium ions, as described by Hayes et al. (Analytical Chem. 66:1860-1865 (1994)) or ferrocyanide as suggested by Roberts and Durst (Analytical Chem. 67:482-491 (1995)) wherein ferrocyanide encapsulated within a liposome is released by addition of a drop of detergent at the detection zone with subsequent electrochemical detection of the released ferrocyanide. Other conventional methods may also be used, as appropriate.

Once the amount of detection agent has been quantified, the amount may then be mapped onto another measurement scale. For example, while the result of the inventive assay may be measured as a density of reflectance (Dr), the result reported may be more meaningful in other units, such as RI (intensity relative to that of a control zone or control zones). Results may also be expressed as the number of copies of analyte present in the measurement volume. The mapping of the amount of analyte detected onto other measurement scales is a preferable embodiment for reporting results of the inventive assay.

For instance, the assay results may be mapped onto a relative scale. Using a relative scale, such as Relative Intensity (RI), for internal control(s), Density of Reflectance (Dr) values may be converted into RI values. In a preferable embodiment, a low control may be assigned an RI value of 1 and a high control may be assigned an RI value of 3, even though the ratio of the absolute Dr values of these controls may be different. In a preferable embodiment, the absolute Dr ratio may be at least about 5:1, while the RI ratio may be about 3:1.

By so doing, changes in individual test strips affecting the absolute Dr value will cause the standard curve to shift up and down a Y-axis, but will have a smaller impact on the RI value plotted along the X-axis. This will systematically damp the variability in the reported result, i.e. will manifest as a "negative gain."

For example, if there is a negative gain between the measured amount of analyte and the reported amount of analyte, then large changes in the measured amount of analyte will be mapped into relatively small changes in the reported amount of analyte. Although the underlying variability of the measured amount of analyte does not change, this method may be of use in certain circumstances. The negative gain effect damps some of the test variability and can be used to improve reproducibility of the reported results of the test as compared to simply reporting Dr.

In addition to reporting the assay results on a continuous scale, either directly as the amount of analyte detected or indirectly as a measurement scale onto which the amount of analyte detected has been mapped, the inventive assays may be used in a "cut-off" style assay. If the detection agent is detected in an analyte binding zone, the amount of detection agent detected may be compared against a cut-off value. A cut-off value is the value above which the test may be considered positive; that is, the analyte of interest is present in the fluid sample to some degree of statistical confidence. Below the cut-off value, the test is generally considered not positive -- either the analyte of interest is not present or the inventive lateral flow assay did not detect its presence. While a cut-off may established based upon a directly measured value, such as the amount of analyte detected, the results may be more meaningful if reported on an indirect, or relative, scale.

A cut-off lateral flow assay is more desirable as the measurement separation between a negative value and a positive value increases. A negative value is the reported value on the continuous scale in the case where the analyte of interest is statistically not present. Conversely, a positive value is the reported value on the continuous scale in the case where the analyte of interest is statistically present. As these values converge, the likelihood reduces of being able to statistically tell positives and negatives apart.

Also desirable is a cut-off lateral flow with increased precision at the cut-off. When there is less variation at the chosen cut-off, it is more likely that a positive can be accurately considered a positive and a negative be accurately considered a negative.

The inventive assay results may be mapped onto either a "relative," discussed above, or an "absolute" scale. Absolute scales are measured in actual physical units, such as number of copies of analyte per milliliter of fluid. Measurement in the absolute scale may be preferable in testing for certain diseases or conditions, such as tests for cancer markers. In such preferable embodiments, the result may be expressed in units, such as ng/ml. Accordingly,

the control zones may have value assigned concentrations of control agent. In an extension of the relative measurement concept, the density of reflectance (DR) values of a series of standards of known analyte concentration may be measured and the intensities relative to the controls (RI values) calculated as previously described. The RI values may then be plotted against analyte concentration to construct a standard curve in which the RI values are assigned concentration values of the analyte of interest. The RI of a sample may then be read on this value assigned standard curve, yielding a result labeled in the desired units.

The single population of analyte binding agents and analyte non-specific agents coupled to detection agent according to the invention offers advantages over two population assays. Two population assays may comprise, in one embodiment, one population of analyte binding agents coupled to detection agents, and another population of analyte non-specific agents coupled to detection agents. As is shown below in the Examples, single population lateral flow assays may be made according to the invention that demonstrate advantages versus similar two population lateral flow assays. These advantages include being able to practice the assay in such a way as to provide a wider measurement separation of negative and positive sample populations, together with increased precision at the cut-off.

Many circumstances may affect the absolute reactivity of lateral flow assays, including, but not limited to, manufacturing-derived variations, operator induced variations, environmentally induced variations and sample effects. With conventional lateral flow assays, any of these variations may act to repress or arguably enhance reactivity of one strip over another, resulting in possible false negative or false positive results. Not controlling for these or other variations may result in significant imprecision, non-reproducibility, lack of sensitivity and lack of specificity of the tests.

Lateral flow assays are also subject to a number of interferences which might affect the absolute amount of binding of either analyte binding agent or

analyte non-specific agent to the control binding zone or the analyte binding zone. Influencing factors may include: 1) variability in the release of the analyte binding agent or the control binding agent from a conjugate pad, 2) device to device variation in the non-specific binding of the analyte binding population to the test strip, 3) variability in the movement of the analyte binding population through or along the test strip during the assay due to variation in the pore size of the test strip or membrane strip materials or non-specific aggregation of the analyte binding agent. Variability of absolute measurements of binding due to these or other factors may therefore be unacceptably high in conventional lateral flow assays.

These sorts of variabilities may be reduced in the practice of the invention. The use of a single population of detection agent coupled to control binding agent and detection agent provides several advantages over conventional lateral flow assays with a population comprising control binding agents and another, separate, population comprising analyte binding agents. First, any portion of the lateral flow assay matrix that has been exposed to the analyte non-specific agent is more likely to have been exposed to the analyte detection agent, as compared to conventional two-population assays. Second, any mechanism that impedes or prevents movement of the analyte non-specific agent along or through the lateral flow matrix is more likely to impede or prevent movement of the analyte detection agent, as compared to conventional two-population assays. Third, the analyte non-specific agent may be chosen so as to reduce the amount of non-specific binding of the analyte detection agent.

Furthermore, reduction of non-specific binding may occur due to modification of hydrophobicity/hydrophilicity profile of the analyte detection matrix. Reduction in aggregation "self-association" of the analyte detection matrix particles, which might hinder movement of matrix along the strip, may also be achieved by choosing an analyte non-specific agent with suitable properties. A final advantage is that, due to the need to prepare fewer reagents, manufacturing costs may be reduced.

Multiple control zones, as disclosed herein, offer a number of potential advantages in the practice of lateral flow assays. Additional control zones may be used to extend the dynamic range of the assay standard curve whether the curve is linear or nonlinear. Multiple control zones may also be used to define whether a prozone or high dose hook effect is present in a given assay. If such an effect is present, then the user can be advised to dilute the sample to unambiguously determine the concentration of the analyte in question

The lateral flow assays according to the invention may find use in a variety of applications. For example, the inventive assays may be used to assay for human diseases, such as infectious diseases, or any other human diseases involving recognizable epitopes (e.g. cancer, autoimmune disease, cardiovascular conditions and pathology). The inventive assays may also be used in veterinary, food testing, agricultural, or fine chemical applications. The lateral flow assays according to the invention may be performed in variety of ways, including use of a lateral flow assay testing apparatus, such as that disclosed in the co-pending patent application of Polito et al., entitled "Method and Apparatus for Performing a Lateral Flow Assay," filed on _____, with an Application Serial No. _____ (Atty. Docket No. 19669-702), hereby incorporated by reference. In a preferable embodiment, the lateral flow assay testing apparatus comprises a ReLIA™ testing apparatus, available from BlackHawk BioSystems (San Ramon, CA).

It will be apparent to those skilled in the art that various modifications and variations can be made in the apparatus and methods of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents. Additionally, the following examples are appended for the purpose of illustrating the claimed invention, and should not be construed so as to limit the scope of the claimed invention.

Examples:

Example 1:

A *Helicobacter pylori* single population conjugate was prepared as follows: Six hundred milliliters of 16 nm gold sol (Frens, G., Nature (London) Phys. Sci. **241**:20 (1973)) were adjusted to pH 9.5 with 100 mM Potassium carbonate. The solution was placed in a polypropylene beaker on a stirring motor. A mixture was then prepared of rabbit anti-DNP and *Helicobacter pylori* extract, containing amounts of each reagent equal to twice that necessary to completely block aggregation of the gold sol by sodium chloride (Geoghegan, W.D. et al., J. Histochem. Cytochem. **25**:1187 (1977)).

The mixture (12 ml) was added to the gold sol with stirring at room temperature and adsorption of the proteins to the gold sold was carried out for ten minutes. At the end of this time, 12 ml of a 0.2 micron filtered solution of Bovine Serum Albumin in deionized water was added and stirring was continued for an additional thirty minutes at room temperature. The colloidal gold conjugate was then placed in 250 ml centrifuge bottles and spun down at room temperature for 30 minutes at 13000 RPM (27000 XG) in the GSA head of a Sorvall RC-5C centrifuge. At the end of this time, the supernatants were removed by aspiration and the colloidal gold conjugate was dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was then diluted to approximately 600 ml with 10 mM sodium borate pH 9.0 containing 0.1% PEG (MW 20,000) (Borate/PEG) and again centrifuged as above. Upon completion of the centrifugation, the supernatants were removed by aspiration and the colloidal gold conjugate was again dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was again diluted to approximately 600 ml with Borate/PEG and centrifuged a final time as above. Upon completion of the

centrifugation, the conjugate was dispersed in the remaining liquid by vortex mixing and sonication and then diluted to approximately 15 ml with Borate/PEG. The conjugate solution was then 0.2 micron filtered and stored at 4° C.

The yield was 15 ml of material with an optical density of 26.64 at 520 nm.

Example 2:

An HIV single population conjugate was prepared as follows: Three hundred milliliters of 16 nm gold sol (Frens, G., Nature (London) Phys. Sci. **241**:20 (1973)) were adjusted to pH 9.5 with 100 mM Potassium carbonate. The solution was placed in a polypropylene beaker on a stirring motor. A mixture was then prepared of rabbit anti-DNP and HIV envelope antigen env-131-Horseradish peroxidase conjugate containing amounts of each reagent equal to twice that necessary to completely block aggregation of the gold sol by sodium chloride (Geoghegan, W.D. et al., J. Histochem. Cytochem. **25**:1187 (1977)).

The mixture (4.5 ml) was added to the gold sol with stirring at room temperature and adsorption of the proteins to the gold sol was carried out for ten minutes. At the end of this time, 6 ml of a 0.2 micron filtered solution of Bovine Serum Albumin in deionized water was added and stirring was continued for an additional thirty minutes at room temperature. The colloidal gold conjugate was then placed in 250 ml centrifuge bottles and spun down at room temperature for 60 minutes at 13000 RPM (27000 XG) in the GSA head of a Sorvall RC-5C centrifuge. At the end of this time, the supernatants were removed by aspiration and the colloidal gold conjugate was dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was then diluted to approximately 300 ml with 10 mM sodium borate pH 9.0 containing 0.1% PEG (MW 20,000)

(Borate/PEG) and again centrifuged as above. Upon completion of the centrifugation, the supernatants were removed by aspiration and the colloidal gold conjugate was again dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was again diluted to approximately 300 ml with 10 mM sodium borate pH 9.0 containing 0.1% PEG (MW 20,000) (Borate/PEG) and centrifuged a final time as above. Upon completion of the centrifugation, the conjugate was dispersed in the remaining liquid by vortex mixing and sonication and then diluted to approximately 10 ml with Borate/PEG. The conjugate solution was then 0.2 micron filtered and stored at 4° C.

The yield was 9.5 ml of material with an optical density of 26.74 at 520 nm.

Example 3:

A control conjugate was prepared as follows: Twelve hundred milliliters of 41 nm gold sol (Frens, G., Nature (London) Phys. Sci. **241**:20 (1973)) were adjusted to pH 9.5 with 100 mM Potassium carbonate. The solution was placed in a polypropylene beaker on a stirring motor. A solution was then prepared of rabbit anti-DNP containing reagent equal to twice that necessary to completely block aggregation of the gold sol by sodium chloride (Geoghegan, W.D. et al., J. Histochem. Cytochem. **25**:1187 (1977)).

The solution (12 ml) was added to the gold sol with stirring at room temperature and adsorption of the proteins to the **gold sol** was carried out for ten minutes. At the end of this time, 24 ml of a 0.2 micron filtered solution of Bovine Serum Albumin in deionized water was added and stirring was continued for an additional thirty minutes at room temperature. The colloidal gold conjugate was then placed in 250 ml centrifuge bottles and spun down at room temperature for 30 minutes at 13000 RPM (27000 XG) in the GSA head

of a Sorvall RC-5C centrifuge. At the end of this time, the supernatants were removed by aspiration and the colloidal gold conjugate was dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was then diluted to approximately 1200 ml with 10 mM sodium borate pH 9.0 containing 0.1% PEG (MW 20,000) (Borate/PEG) and again centrifuged as above. Upon completion of the centrifugation, the supernatants were removed by aspiration and the colloidal gold conjugate was again dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was again diluted to approximately 1200 ml with Borate/PEG and centrifuged a final time as above. Upon completion of the centrifugation, the conjugate was dispersed in the remaining liquid by vortex mixing and sonication and then diluted to approximately 42 ml with Borate/PEG. The conjugate solution was then 0.2 micron filtered and stored at 4° C.

The yield was 42 ml of material with an optical density of 11.76 at 520 nm.

Example 4:

A *Helicobacter pylori* conjugate was prepared as follows: One hundred forty milliliters of 16 nm gold sol (Frens, G., Nature (London) Phys. Sci. **241**:20 (1973)) were adjusted to pH 9.5 with 100 mM Potassium carbonate. The solution was placed in a polypropylene beaker on a stirring motor. A solution was then prepared of *Helicobacter pylori* extract containing reagent equal to twice that necessary to completely block aggregation of the gold sol by sodium chloride and nonspecific rabbit IgG equal to twice that necessary to completely block sodium chloride induced aggregation of the gold sol. Geoghegan, W.D. et al., J. Histochem. Cytochem. **25**:1187 (1977).

The solution (2.8 ml) was added to the gold sol with stirring at room temperature and adsorption of the proteins to the gold sold was carried out for ten minutes. At the end of this time, 2.8 ml of a 0.2 micron filtered solution of Bovine Serum Albumin in deionized water was added and stirring was continued for an additional thirty minutes at room temperature. The colloidal gold conjugate was then placed in 250 ml centrifuge bottles and spun down at room temperature for 30 minutes at 13000 RPM (27000 XG) in the GSA head of a Sorvall RC-5C centrifuge. At the end of this time, the supernatants were removed by aspiration and the colloidal gold conjugate was dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was then diluted to approximately 140 ml with 10 mM sodium borate pH 9.0 containing 0.1% PEG (MW 20,000) (Borate/PEG) and again centrifuged as above. Upon completion of the centrifugation, the supernatants were removed by aspiration and the colloidal gold conjugate was again dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was again diluted to approximately 140 ml with Borate/PEG and centrifuged a final time as above. Upon completion of the centrifugation, the conjugate was dispersed in the remaining liquid by vortex mixing and sonication and then diluted to approximately 5 ml with Borate/PEG. The conjugate solution was then 0.2 micron filtered and stored at 4° C.

The yield was 4.5 ml of material with an optical density of 28.4 at 520 nm.

Example 5:

An HIV detection conjugate was prepared as follows: Four hundred milliliters of 16 nm gold sol (Frens, G., Nature (London) Phys. Sci. **241**:20 (1973)) were adjusted to pH 9.5 with 100 mM Potassium carbonate. The

solution was placed in a polypropylene beaker on a stirring motor. A solution was then prepared of HIV envelope antigen env-131-Horseradish peroxidase conjugate containing reagent equal to twice that necessary to completely block aggregation of the gold sol by sodium chloride and nonspecific rabbit IgG containing twice that necessary to completely block sodium chloride induced aggregation of the gold sol (Geoghegan, W.D. et al., J. Histochem. Cytochem. 25:1187 (1977)).

The solution (4.26 ml) was added to the gold sol with stirring at room temperature and adsorption of the proteins to the gold sol was carried out for ten minutes. At the end of this time, 8 ml of a 0.2 micron filtered solution of Bovine Serum Albumin in deionized water was added and stirring was continued for an additional thirty minutes at room temperature. The colloidal gold conjugate was then placed in 250 ml centrifuge bottles and spun down at room temperature for 30 minutes at 13000 RPM (27000 XG) in the GSA head of a Sorvall RC-5C centrifuge. At the end of this time, the supernatants were removed by aspiration and the colloidal gold conjugate was dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was then diluted to approximately 400 ml with 10 mM sodium borate pH 9.0 containing 0.1% PEG (MW 20,000) (Borate/PEG) and again centrifuged as above. Upon completion of the centrifugation, the supernatants were removed by aspiration and the colloidal gold conjugate was again dispersed in the remaining liquid by vortex mixing and sonication.

The colloidal gold conjugate was again diluted to approximately 400 ml with Borate/PEG and centrifuged a final time as above. Upon completion of the centrifugation, the conjugate was dispersed in the remaining liquid by vortex mixing and sonication and then diluted to approximately 15 ml with Borate/PEG. The conjugate solution was then 0.2 micron filtered and stored at 4° C.

The yield was 12 ml of material with an optical density of 26.66 at 520 nm.

Example 6:

Test strips were manufactured according to the following procedure. Backed sheets of Millipore STHF nitrocellulose 2.5 cm X 20 cm were coated by longitudinally dispensing one antigen and two control bands onto the nitrocellulose using a Bio Dot XYZ3000 dispensing platform with Biojets operating at a frequency of 120 Hz, 20.83 nl/drop, and 0.5 ul/cm. The control antigen was Dinitrophenylated Bovine Serum Albumin (DNP-BSA) in Phosphate buffered saline (PBS) containing 0.05% Tween 20. High control was coated in the range of 150 - 500 ug/ml and low control was coated in the range of 15 - 100 ug/ml as appropriate to the individual assay. Antigens were coated in the range of 0.5 - 4 mg/ml, as appropriate to the individual assay, in phosphate buffered saline containing detergent. Reducing agent, such as DTT and/or ethylenediamine tetraacetic acid (EDTA) were added to the antigen solution if active sulfhydryl groups were present. In the strips used in example 23, HIV env-131 antigen was coated at 2 mg/ml in PBS, 2 mM EDTA, 10 mM DTT, 0.2% sodium dodecyl sulfate (SDS), high control coating concentration was 300 ug/ml and low control coating concentration was 25 ug/ml.

Sheets were then dried for one hour at 37 °C for one hour in a forced air incubator, blocked for fifteen minutes in a solution of PBS containing 10 mg/ml BSA, 1% (w/v) PEG 8000, 3% (w/v) mannitol, 0..3% (w/v) gelatin, 0.01% (w/v) sodium azide and 0.05% (w/v) sodium dodecyl sulfate, and then dried for an additional hour in a forced air incubator at 37 °C. Coated sheets were stored desiccated at room temperature in foil pouches.

Gelman 8980 glass fiber pads were preblocked by dipping in a solution of PBS 10 mg/ml BSA, 2 mg/ml rabbit IgG, 1% (w/v) Triton X-100, 2.5% (w/v) sucrose and 0.3% (w/v) polyvinylpyrrolidone K-30 and then drying for

1.5 hours in a forced air incubator. The preblocked conjugate pads were coated with either HIV OMNI™ conjugate, made according to Example 2, or *Helicobacter pylori* OMNI™ conjugate, made according to Example 1, by mixing equal volumes of the stock conjugate solution (OD 520 approximately 26) with PBS 20 mg/ml BSA, 4 mg/ml rabbit IgG, 2% (w/v) Triton X-100, 5% (w/v) sucrose and 0.6% (w/v) polyvinylpyrrolidone K-30. 1/40th volume of 20x PBS was then added and the solution 0.2 micron filtered and degassed at 27 inches of mercury vacuum for one hour at room temperature. The conjugate mix was then longitudinally dispensed on the preblocked conjugate pads using a Bio Dot XYZ3000 Dispensing platform with a single Biojet operating at a frequency of 120 Hz and delivering 104.17 nl/drop and 2.5 ul/cm. The conjugate was coated in patterns of four lines per cm with three patterns coated on each 3 cm X 10 cm pad. Coated pads were vacuum dried at 2 Torr for one hour at room temperature and then cut into three 1 cm X 10 cm sections each containing one four line pattern.

Test strips were prepared by affixing one 2.5 cm X 20 cm backed nitrocellulose sheet, one 1.8 cm X 20 cm sheet of Gelman Type 133 Absorbent Pad, two 1 cm X 10 cm conjugate coated pads (side by side) and one 2 cm X 20 cm Gelman Cytosep 1662 sheet to one adhesive coated .010" thick 6 cm X 20 cm vinyl backing sheet (G&L Precision Die Cutting) in the configuration shown in FIG. 1. Clear adhesive backed polyester film (G&L Precision Die Cutting) was used to cover the test strip from the top 2 mm of the cytosep to the top of the absorbent pad. Strips 0.5 cm wide were cut from the assembled sheet with a Bio Dot Cutter 3000™.

Example 7:

Strips used in this example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori*

extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control zone and the high control zone farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 0.968 ml of the conjugate described in Example 3, 0.681 ml of the conjugate described in Example 4, 1.868 ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG, 0.094 ml 20X PBS and 0.185 ml of 10 mM Borate pH 9.0, 0.1% PEG (MW 20000). The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul *H. pylori* negative adsol plasma sample (n=12) followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C. Strips were read after 15 minutes.

The results were as follows:

Sample	HC(DR)	LC(DR)	Specimen(DR)	HC/LC	RI
Avg.	.1565	.0253	.0230	6.3994	.9195
SD	.0149	.0052	.0060	1.1969	.2005
CV	9.5%	20.4%	26.1%	18.7%	21.8%

Example 8:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 1.0 ml of the conjugate described in Example 1, 1.0.ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG and 0.050 ml 20X PBS The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul *H. pylori* negative adsol sample (n=12) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C. Strips were read after 15 minutes.

The results were as follows:

Sample	HC(DR)	LC(DR)	Specimen(DR)	HC/LC	RI
Avg.	.2120	.0370	.0568	5.8153	1.2201
SD	.0322	.0079	.0167	.6194	.1252
CV	15.2%	21.5%	29.5%	10.6%	10.3%

Example 9:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori*

extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 0.968 ml of the conjugate described in Example 3, 0.681 ml of the conjugate described in Example 4, 1.868 ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG, 0.094 ml 20X PBS and 0.185 ml of 10 mM Borate pH 9.0, 0.1% PEG (MW 20000). The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul *H. pylori* negative adsol plasma sample (n=12) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	HC	LC	Specimen	HC/LC	RI
Avg.	.1763	.0441	.0069	4.0048	.1595
SD	.0192	.0071	.0047	.3692	.1182
CV	10.9%	16.0%	68.8%	9.2%	74.1%

Example 10:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori*

extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was analyte binding (i.e. antigen) zone closest to the conjugate pad, low control zone between the analyte binding (i.e. antigen) zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 1.0 ml of the conjugate described in Example 1, 1.0.ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG and 0.050 ml 20X PBS. The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul *H. pylori* negative adsol plasma sample (n=12) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results are as follows:

Sample	HC(DR)	LC(DR)	Specimen(DR)	HC/LC	RI
Avg.	.2176	.0257	.0176	8.6990	.6957
SD	.0251	.0056	.0052	1.2565	.2309
CV	11.5%	21.8%	29.3%	14.4%	33.2%

Example 11:

Strips used in this example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25

ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control zone and the high control zone farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 0.968 ml of the conjugate described in Example 3, 0.681 ml of the conjugate described in Example 4, 1.868 ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG, 0.094 ml 20X PBS and 0.185 ml of 10 mM Borate pH 9.0, 0.1% PEG (MW 20000). The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul BBI PMH 201 Mixed Titer Panel (n=7) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	HC(DR)	LC(DR)	HC/LC		
Avg.	.1457	.0226	6.6858		
SD	.0138	.0046	1.4142		
CV	9.5%	20.2%	21.1%		

Example 12:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 1.0 ml of the conjugate described in Example 1, 1.0.ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG and 0.050 ml 20X PBS The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul BBI PMH 201 Mixted Titer Panel (n=7) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	HC(DR)	LC(DR)	HC/LC
Avg.	.1912	.0307	6.3801
SD	.0403	.0086	.8666
CV	21.0%	28.1%	13.6%

Example 13:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 0.968 ml of the conjugate described in Example 3, 0.681 ml of the conjugate described in Example 4, 1.868 ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG, 0.094 ml 20X PBS and 0.185 ml of 10 mM Borate pH 9.0, 0.1% PEG (MW 20000). The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul BBI PMH 201 Mixed Titer Panel (n=7) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were follows:

Sample	HC(Dr)	LC(DR)	HC/LC
Avg.	.1676	.0429	3.9503
SD	.0163	.0067	.3697
cv	9.7%	15.6%	9.4%

Example 14:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was analyte binding (i.e. antigen) zone closest to the conjugate pad, low control zone between the analyte binding (i.e. antigen) zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 1.0 ml of the conjugate described in Example 1, 1.0.ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG and 0.050 ml 20X PBS The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul BBI PMH Mixed Titer Panel (n=7) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	HC(DR)	LC(DR)	HC/LC
Avg.	.1881	.0238	8.0320
SD	.0289	.0053	.9351
CV	15.4%	22.2%	11.6%

Example 15:

Strips used in this example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control zone and the high control zone farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 0.968 ml of the conjugate described in Example 3, 0.681 ml of the conjugate described in Example 4, 1.868 ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG, 0.094 ml 20X PBS and 0.185 ml of 10 mM Borate pH 9.0, 0.1% PEG (MW 20000). The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul random sera (n=10) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	HC(DR)	LC(DR)	HC/LC
Avg.	.1452	.0250	6.0055
SD	.0211	.0067	.9374
CV	14.6%	26.9%	15.6%

Example 16:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 1.0 ml of the conjugate described in Example 1, 1.0.ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG and 0.050 ml 20X PBS The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul random sera (n=10) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	HC(DR)	LC(DR)	HC/LC
Avg.	.1913	.0335	5.7668
SD	.0274	.0054	.7525
CV	14.3%	16.2%	13.0%

Example 17:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori*

extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 0.968 ml of the conjugate described in Example 3, 0.681 ml of the conjugate described in Example 4, 1.868 ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG, 0.094 ml 20X PBS and 0.185 ml of 10 mM Borate pH 9.0, 0.1% PEG (MW 20000). The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul random sera (n=10) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	HC	LC	HC/LC
Avg.	.1716	.0431	4.0028
SD	.0152	.0048	.2896
CV	8.9%	11.1%	7.2%

Example 18:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori*

extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was analyte binding (i.e. antigen) zone closest to the conjugate pad, low control zone between the analyte binding (i.e. antigen) zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 1.0 ml of the conjugate described in Example 1, 1.0.ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG and 0.050 ml 20X PBS The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul random sera (n=10) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	HC(Dr)	LC(DR)	HC/LC
Avg.	2.089	.0224	9.7132
SD	.0296	.0065	1.7922
CV	14.2%	28.8%	18.4%

Example 19:

Strips used in this example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e.

antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control zone and the high control zone farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 0.968 ml of the conjugate described in Example 3, 0.681 ml of the conjugate described in Example 4, 1.868 ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG, 0.094 ml 20X PBS and 0.185 ml of 10 mM Borate pH 9.0, 0.1% PEG (MW 20000). The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul sample (as indicated below) followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	N	HC(DR)	LC(DR)	Specimen(DR)	HC/LC	RI	S/CO
90528 (Neg Serum)	4						
Avg.		.1793	.0309	.0254	6.0867	.8439	.65
SD		.0313	.0095	.0040	1.3917	.2141	
CV		17.4%	30.7%	15.6%	23.0%	25.4%	
PMH201-04 (Pos)	4						
Avg.		.1607%	.0267	.0655	6.3236	1.5765	1.21
SD		.0154	.0076	.0152	1.5435	.1030	
CV		9.6%	28.6%	23.2%	24.4%	6.5%	

Example 20:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 1.0 ml of the conjugate described in Example 1, 1.0.ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG and 0.050 ml 20X PBS The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul sample (as described below) followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	N	HC(DR)	LC(DR)	Specimen(DR)	HC/LC	RI	S/CO
90530 (Neg Serum)	4						
Avg.		.1940	.0345	.0400	5.6471	1.0685	.71
SD		.0008	.0026	.0088	.4166	.1235	
CV		.4%	7.7%	22.1%	7.4%	11.6%	
PMH201-02 (+/-)	4						
Avg.		.2006	.0340	.0768	5.9357	1.5117	1.01
SD		.0220	.0044	.0091	.6142	.0639	
CV		11.0%	13.0%	11.8%	10.3%	4.2%	
PMH201-11 (Pos)	4						
Avg.		.1755	.0453	.2420	3.9757	4.0194	2.68
SD		.0180	.0097	.0281	.6497	.1078	
CV		10.2%	21.5%	11.6%	16.3%	2.7%	

Example 21:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was low control zone closest to the conjugate pad, analyte binding (i.e. antigen) zone between the low control zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 0.968 ml of the conjugate described in Example 3, 0.681 ml of the conjugate described in Example 4, 1.868 ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG, 0.094 ml 20X PBS and 0.185 ml of 10 mM Borate pH 9.0, 0.1% PEG (MW 20000). The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt,

the assay was commenced by adding a 25 ul BBI PMH Mixed Titer Panel (n=4) sample followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	HC	LC	Specimen	HC/LC	RI	S/CO
Avg.	.1723	.0410	.0182	4.2373	4.378	1.09
SD	.0107	.0050	.0052	.4514	.0849	
CV	6.2%	12.2%	28.7%	10.6%	19.4%	

Example 22:

Strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a solution of *Helicobacter pylori* extract having an optical density at 280 nm of 2.70 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was analyte binding (i.e. antigen) zone closest to the conjugate pad, low control zone between the analyte binding (i.e. antigen) zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6. Conjugate pads were prepared by mixing 1.0 ml of the conjugate described in Example 1, 1.0.ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG and 0.050 ml 20X PBS The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assay was carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the *H. pylori* assay. At the prompt, the assay was commenced by adding a 25 ul sample (as described below) followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG

(20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C Strips were read after 15 minutes.

The results were as follows:

Sample	N	HC(DR)	LC(DR)	Specimen(DR)	HC/LC	RI	S/CO
90530 (Neg Serum)	4						
Avg.		.2097	.0253	.0081	8.4425	.0359	.2549
SD		.0172	.0048	.0058	1.0962	.1997	
CV		8.2%	19.1%	72.0%	13.0%	65.3%	
PMH201-02 (+/-)	4						
Avg.		.2175	.0253	.0330	8.6525	1.0800	.9000
SD		.0113	.0021	.0026	.8507	.0198	
CV		5.2%	8.2%	7.9%	9.8%	1.8%	
PMH201-11 (Pos)	4						
Avg.		.1556	.0146	.1209	10.7818	2.5104	2.09
SD		.0035	.0018	.0097	1.4863	.1592	
CV		2.3%	12.5%	8.0%	13.8%	6.3%	

The data from the above Examples may be summarized as follows. Four assay formats were tested in Examples 7 through 22. The four assay formats were:

Format 1: separate conjugate populations of particles having control binding agent and particles having analyte binding agent, with the analyte binding zone placed between a high control zone and a low control zone;

Format 2: separate conjugate populations of particles having control binding agent and particles having analyte binding agent, with the high control zone and low control zone placed after the analyte binding zone place and the conjugate pad;

Format 3: OMNI™ conjugate populations of particles comprising control binding agent and analyte binding agent, with the analyte binding zone placed between a high control zone and a low control zone; and

Format 4: OMNI™ conjugate populations of particles comprising control binding agent and analyte binding agent, with the high control zone and low control zone placed after the analyte binding zone place and the conjugate pad

The results are analyzed by Format in Tables 1 to 5.

Table 1:

Reported as %CV of HC/LC

Specimen	Format 1	Format 2	Format 3	Format 4
Adsol H. pylori neg plasmas	18.7%	9.2%	10.6%	14.4%
BBI PMH201 Mixed titer panel (sera/plasmas)	21.1%	8.7%	13.6%	11.6%
Random sera	15.6%	7.2%	13.0%	18.4%
Avg	18.5%	8.4%	12.4%	14.8%

Table 2:

Results for Negative Specimens, reported as Avg Dr (Analyte Binding Zone)/ Avg. Dr Low Control Zone:

Specimen	Format 1	Format 2	Format 3	Format 4
Adsol Plasmas	0.91	0.16	1.64	0.68
BBI PMH201-3	1.04	0.27	1.96	0.49
Random sera	0.40	0.03	0.84	0.57
Avg	0.78	0.15	1.45	0.58

Table 3:

Results on Positive Specimens, reported as Avg. Dr (Analyte Binding Zone)/ Avg Dr Low Control Zone:

Specimen	Format 1	Format 2	Format 3	Format 4
PMH201-02	1.76	0.52	2.74	2.10
PMH201-04	2.51	0.34	4.33	1.42
PMH201-08	1.38	0.50	2.16	1.25
0290531	2.27	0.98	4.29	4.50
0290532	1.15	0.40	2.06	2.20
0290534	2.18	0.88	5.91	4.33
Avg.	1.87	0.60	3.58	2.63

Table 4:

Population Spread, reported as Table 3 Avg./Table 2 Avg.

	Format 1	Format 2	Format 3	Format 4
Ratio Pos/Neg	2.40	4.0	2.47	4.53

Table 5:

Assay precision at the cutoff (S/CO 0.9-1.2)

Format	Specimen	Avg (N=4) S/CO	% CV
Format (1)	PMH201-04	1.21	6.5%
Format (2)	PMH201-08	1.09	19.4%
Format (3)	PMH201-02	1.01	4.2
Format (4)	PMH201-04	0.90	1.8%

As can be seen from the Tables, different arrangements of individual particle populations versus OMNI™ conjugate populations, and different arrangements of control and analyte binding zones produces different results. For the purposes of the *H. pylori* assay, Format 2 produces the lowest coefficients of variation on the HC/LC ratio and the lowest readings for *H. pylori*-negative samples. In contrast, Format 3 produces the highest readings for *H. pylori*-positive samples. Finally, Format 4 produces the best precision at the designated cut-off value, and also has the greatest spread between measured values for *H. pylori*-negative samples and *H. pylori*-positive samples.

Format 4 therefore appears to be the best choice for an *H. pylori* test using a cut-off approach.

Example 23:

Format 2 and format 4 strips used in this Example were coated with 300 ug/ml Dinitrophenyl conjugated bovine serum albumin (BSA-DNP) in the high control zone, 25 ug/ml BSA-DNP in the low control zone and a 2 mg/ml solution of HIV envelope antigen env-131 in the analyte binding (i.e. antigen) zone. The order of the bands on the strip was analyte binding (i.e. antigen) zone closest to the conjugate pad, low control zone between the analyte binding (i.e. antigen) zone and the high control and the high control farthest from the conjugate pad and closest to the absorbent pad. Nitrocellulose sheets were coated and strips prepared as described in Example 6.

For format 2 strips, conjugate pads were preblocked in 0.1M sodium phosphate pH 7.4 containing 0.5 M sodium chloride, 1% (w/v) casein, 1 mM EDTA, 1% (w/v) Triton X-100, 0.1% (w/v) sodium azide, 0.05% (w/v) Gentamycin sulfate, 0.1% (w/v) yeast extract, 0.05% (w/v) *E. coli* extract, 1% (w/v) BSA, 0.3% polyvinylpyrrolidone, 0.05 % SDS, 2.5% sucrose and 2 mg/ml rabbit IgG and dried for 1.5 hours at 37 °C. Conjugate mixture was prepared by mixing 0.908 ml of the conjugate described in Example 3, 1.440 ml of the conjugate mixture described in Example 5 and 1.388 ml of 10 mM Borate pH 9.0, 0.1% PEG (MW 20000). The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

For format 4 strips, conjugate pads were prepared by mixing 1.0 ml of the conjugate described in Example 2, 1.0.ml of PBS 20 mg/ml BSA, 2% Triton X-100, 5% sucrose, 0.6% PVP K-30 and 4 mg/ml rabbit IgG and 0.050 ml 20X PBS. The mixture was dispensed onto preblocked conjugate pads as described in Example 6.

The assays were carried out by placing the cassette containing the strip in a ReLIA™ machine set up to run and read the HIV assay. At the prompt, the assay was commenced by adding a 25 ul sample (either HIV+ or HIV-) followed by 3 drops of wash buffer (PBS, 0.05% Triton X-100, 0.5% PEG (20K), 0.1% sodium azide and 2 mM EDTA). Strip temperature was set to 37 °C. Strips were read after 15 minutes. The results were as follows:

Sample	Dr	Format (4) CO = 1.0 RI	S/CO	DR	Format (2) CO = 0.15 RI	S/CO	Abbott1st Gen HIV-1 S/CO	Cambridge Western Blot
P RB106-02	0.0166	1.0525	1.05	0.0044	0.0830	0.54	0.2	Neg
PRB106-06	0.0104	0.7089	0.71	0.0049	0.0975	0.46	0.2	Neg
PRB106-11	0.1213	2.2926	2.29	0.0035	0.0493	0.34	6.2	Pos
PRB106-12	0.0546	1.4083	1.41	0.0068	0.1222	0.62	3.8	Pos
Sample #2 is the same donor as sample #12, obtained 2 days earlier								
PRB203-03	0.0118	0.6559	0.66	0.0000	0.0000	0.00	0.2	Neg
PRB203-07	0.0597	1.4626	1.46	0.0044	0.0859	0.54	2.4	Pos
PRB203-14	0.0656	1.6134	1.61	0.0094	0.1598	1.04	0.4	Neg
PRB203-24	0.1174	2.2679	2.26	0.0018	0.1961	1.29	2.4	Pos
Sample #14 is the same donor as sample #24, obtained 9 days earlier								

In the above Examples, use of a single population of detection agent couple to an analyte binding agent and a control binding agent showed improvement over the current state of the art, represented by two separate populations. An advantage of the inventive single population embodiment format is that it may improve the separation of negative from positive sample populations. The single population embodiment may also improve the precision around the cutoff by reducing nonspecific binding and providing an improved flow control mechanism. The result may be improved assay sensitivity and specificity compared to two population embodiments.

In addition, the use of multiple control zones and the ability to express results as intensity relative to the control bands helps to improve assay reproducibility and precision at the cutoff. This may be accomplished in the inventive single population embodiment by improved control of sources of assay variability compared to separate populations of first analyte detection and control detection particles. Of course, while embodiments comprising single

populations of first analyte binding agents and analytes non-specific agents coupled to detection agents may prove superior under certain circumstances, the present invention encompasses embodiments that may possess multiple (two or more) populations of first analyte binding agents and analytes non-specific agents coupled to detection agents.

The order of placement of the control binding zones and analyte binding zones on the strip relative to the direction of flow appeared to affect assay performance in the above Examples. Moving from the conjugate pad to the absorbent pad, the optimum assay configuration appeared to be analyte binding (i.e. antigen) zone, low control zone and high control zone for both the *Helicobacter pylori* and HIV assays. However, this does not mean that optimum results cannot be obtained by different zone configurations in other assays, and the present invention specifically encompasses such other arrangements.

WHAT IS CLAIMED IS:

1. A test strip adapted to receive a sample and evaluate an analyte therein, the test strip comprising:

an application zone to which a sample may be added;

a conjugate zone comprising an omni conjugate agent having bound thereto a first analyte binding agent which is capable of binding to the analyte to be detected, a control binding agent, and a detection agent;

an analyte measurement zone including a second analyte binding agent immobilized therein which is capable of binding to the analyte to be detected; and

one or more control measurement zones, each control zone including a control agent immobilized therein which is capable of binding to the control binding agent;

wherein during operation of the test strip, a sample added to the application zone diffuses from the conjugate zone to the analyte measurement zone and the one or more control measurement zones.

2. The test strip according to claim 1 wherein the analyte that the test strip is designed to detect is selected from the group consisting of polypeptides, polysaccharides, polynucleic acids, small molecules, toxins, drugs, viruses, and virus particles.

3. The test strip according to claim 1, wherein the analyte is a molecule selected from the group consisting of small organic molecules, antibodies or portions thereof, nucleic acids, and portions of a cell wall.

4. The test strip according to claim 1, wherein the analyte is a molecule selected from the group consisting of hormones, secreted proteins, enzymes, and cell surface proteins.

5. The test strip according to claim 1, wherein the analyte is a molecule selected from the group consisting of monoclonal and polyclonal antibodies.

6. The test strip according to claim 1, wherein the analyte comprises an immunogenic portion.

7. The test strip according to claim 1, wherein the analyte binding agent is selected from the group consisting of antibodies, engineered proteins, peptides, haptens, lysates containing heterogeneous mixtures of antigens having analyte binding sites, ligands and receptors.

8. The test strip according to claim 1, wherein the analyte binding agent does not bind to components in the sample other than the analyte.

9. The test strip according to claim 1 wherein the detection agent is selected from the group consisting of fluorescent, colorimetric, luminescent, chemical label, enzyme, radioactive, and radiofrequency detectable markers.

10. The test strip according to claim 1 wherein the detectable marker is capable of being measured by reflectance.

11. The test strip according to claim 1 wherein the control binding agent is attached to a particle which is capable of diffusing to the analyte measurement zone.

12. The test strip according to claim 1 wherein the one or more control measurement zones include a first control measurement zone and a second control measurement zone.

13. The test strip according to claim 12 wherein the first control measurement zone and the second control measurement zone are spaced from each other on the test strip.

14. The test strip according to claim 1, wherein at least one of the first analyte binding agent and the control binding agent are covalently coupled to the detection agent.

15. The test strip according to claim 1, wherein at least one of the first analyte binding agent and the control binding agent are non-covalently coupled to the detection agent.

16. The test strip according to claim 1, wherein the detection agent comprises particles.

17. The test strip according to claim 1, wherein the detection agent comprises colloidal gold.

18. The test strip according to claim 1, wherein the control agent is selected from the group consisting of an antibody, an engineered protein having binding sites non-specific for the analyte, a receptor that specifically binds to ligands other than the analyte, a ligand that specifically binds to receptors other than the analyte, an antigen, organic molecule, a hapten, lgG, immunoglobulins, albumins, casein, and globulin.

19. The test strip according to claim 1, wherein the control agent comprises rabbit IgG.

20. The test strip according to claim 1, wherein the control agent comprises rabbit anti-dinitrophenol IgG.

21. The test strip according to claim 1, wherein the control agent comprises dinitrophenol.

22. The test strip according to claim 1, wherein the one or more control measurement zones are positioned spatially apart from the analyte measurement zone on the test strip.

23. A test strip adapted to receive a sample and evaluate two analytes therein, the test strip comprising:

an application zone to which a sample may be added;

a conjugate zone comprising

a first omni conjugate agent having bound thereto a first analyte binding agent which is capable of binding to the first analyte to be detected, a control binding agent, and a detection agent, and

a second omni conjugate agent having bound thereto a third analyte binding agent which is capable of binding to the second analyte to be detected, a control binding agent, and a detection agent;

a first analyte measurement zone including a second analyte binding agent immobilized therein which is capable of binding to the first analyte to be detected;

a second analyte measurement zone including a fourth analyte binding agent immobilized therein which is capable of binding to the second analyte to be detected; and

one or more control measurement zones, each control zone including a control agent immobilized therein which is capable of binding to the control binding agent;

wherein during operation of the test strip, a sample added to the application zone diffuses from the conjugate zone to the first and second analyte measurement zones and the one or more control measurement zones.

24. A method for evaluating an analyte in a sample comprising:

contacting the sample containing the analyte with an omni conjugate agent having bound thereto a first analyte binding agent which is capable of binding to the analyte to be detected, a control binding agent, and a detection agent;

diffusing analyte which has become bound to the omni conjugate agent to an analyte measurement zone which includes a second analyte binding agent immobilized therein, the second analyte binding agent binding to and immobilizing analyte which is bound to the omni conjugate agent to cause detection agent to be immobilized in the analyte measurement zone;

diffusing the omni conjugate agent to one or more control zones which each includes a control agent immobilized therein, the control binding agent binding to and immobilizing control binding agent which is bound to the omni conjugate agent to cause detection agent to be immobilized in the one or more control zones; and

measuring the detection agent immobilized in the analyte measurement zone and the one or more control zones.

25. The method according to claim 24 wherein the analyte detected is selected from the group consisting of polypeptides, polysaccharides, polynucleic acids, small molecules, toxins, drugs, viruses, and virus particles.

26. The method according to claim 24 wherein the analyte detected is a molecule selected from the group consisting of small organic molecules, antibodies or portions thereof, nucleic acids, and portions of a cell wall.

27. The method according to claim 24 wherein the analyte detected is a molecule selected from the group consisting of hormones, secreted proteins, enzymes, and cell surface proteins.

28. The method according to claim 24 wherein the analyte detected is a molecule selected from the group consisting of monoclonal and polyclonal antibodies.

29. The method according to claim 24 wherein the analyte detected comprises an immunogenic portion.

30. The method according to claim 24 wherein the analyte binding agent is selected from the group consisting of antibodies, engineered proteins, peptides, haptens, lysates containing heterogeneous mixtures of antigens having analyte binding sites, ligands and receptors.

31. The method according to claim 24 wherein the analyte binding agent does not bind to components in the sample other than the analyte.

32. The method according to claim 24 wherein the detection agent is selected from the group consisting of fluorescent, colorimetric, luminescent, chemical label, enzyme, radioactive, and radiofrequency detectable markers.

33. The method according to claim 24 wherein the detectable marker is capable of being measured by reflectance.

34. The method according to claim 24 wherein the control binding agent is attached to a particle which is capable of diffusing to the analyte measurement zone.

35. The method according to claim 24 wherein the one or more control measurement zones include a first control measurement zone and a second control measurement zone.

36. The method according to claim 35 wherein the first control measurement zone and the second control measurement zone are spaced from each other on the test strip.

37. The method according to claim 24 wherein at least one of the first analyte binding agent and the control binding agent are covalently coupled to the detection agent.

38. The method according to claim 24 wherein at least one of the first analyte binding agent and the control binding agent are non-covalently coupled to the detection agent.

39. The method according to claim 24 wherein the detection agent comprises particles.

40. The method according to claim 24 wherein the detection agent comprises colloidal gold.

41. The method according to claim 24 wherein the control agent is selected from the group consisting of an antibody, an engineered protein having binding sites non-specific for the analyte, a receptor that specifically binds to ligands other than the analyte, a ligand that specifically binds to receptors other than the analyte, an antigen, organic molecule, a hapten, lgG, immunoglobulins, albumins, casein, and globulin.

42. The method according to claim 24 wherein the control agent comprises rabbit IgG.

43. The method according to claim 24 wherein the control agent comprises rabbit anti-dinitrophenol IgG.

44. The method according to claim 24 wherein the control agent comprises dinitrophenol.

45. The method according to claim 24 wherein the analyte measurement zone and the one or more control zones are positioned on a lateral flow test strip, the method being performed on the lateral flow test strip.

46. The method according to claim 45 wherein the one or more control measurement zones are positioned spatially apart from the analyte measurement zone on the test strip.

47. A test strip adapted to receive a sample and evaluate analytes therein, the test strip comprising:

an application zone to which a sample may be added;

a conjugate zone comprising an omni conjugate agent having bound thereto a first analyte binding agent which is capable of binding to a first analyte to be detected, a third analyte binding agent which is capable of binding to a second analyte to be detected, a control binding agent, and a detection agent;

a first analyte measurement zone including a second analyte binding agent immobilized therein which is capable of binding to the first analyte to be detected;

a second analyte measurement zone including a fourth analyte binding agent immobilized therein which is capable of binding to the second analyte to be detected; and

one or more control measurement zones, each control zone including a control agent immobilized therein which is capable of binding to the control binding agent;

wherein during operation of the test strip, a sample added to the application zone diffuses from the conjugate zone to the first and second analyte measurement zones and the one or more control measurement zones.

48. A test strip adapted to receive a sample and evaluate an analyte therein, the test strip comprising:

an application zone to which a sample may be added;

a conjugate zone comprising a first analyte binding agent which is capable of binding to the analyte to be detected and a control binding agent, the first analyte binding agent and the control binding agent each having a detection agent bound thereto;

an analyte measurement zone including a second analyte binding agent immobilized therein which is capable of binding to the analyte to be detected; and

one or more control measurement zones, each control zone including a control agent immobilized therein which is capable of binding to the control binding agent, the one or more control measurement zones being positioned spatially apart from the analyte measurement zone on the test strip;

wherein during operation of the test strip, a sample added to the application zone diffuses from the conjugate zone to the analyte measurement zone and the one or more control measurement zones.

49. The test strip according to claim 48 wherein the analyte that the test strip is designed to detect is selected from the group consisting of polypeptides, polysaccharides, polynucleic acids, small molecules, toxins, drugs, viruses, and virus particles.

50. The test strip according to claim 48, wherein the analyte is a molecule selected from the group consisting of small organic molecules, antibodies or portions thereof, nucleic acids, and portions of a cell wall.

51. The test strip according to claim 48, wherein the analyte is a molecule selected from the group consisting of hormones, secreted proteins, enzymes, and cell surface proteins.

52. The test strip according to claim 48, wherein the analyte is a molecule selected from the group consisting of monoclonal and polyclonal antibodies.

53. The test strip according to claim 48, wherein the analyte comprises an immunogenic portion.

54. The test strip according to claim 48 wherein the analyte binding agent is selected from the group consisting of antibodies, engineered proteins, peptides, haptens, lysates containing heterogeneous mixtures of antigens having analyte binding sites, ligands and receptors.

55. The test strip according to claim 48 wherein the analyte binding agent does not bind to components in the sample other than the analyte.

56. The test strip according to claim 48 wherein the detection agent is selected from the group consisting of fluorescent, colorimetric, luminescent, chemical label, enzyme, radioactive, and radiofrequency detectable markers.

57. The test strip according to claim 48 wherein the detectable marker is capable of being measured by reflectance.

58. The test strip according to claim 48 wherein the control binding agent is attached to a particle which is capable of diffusing to the analyte measurement zone.

59. The test strip according to claim 48, wherein at least one of the first analyte binding agent and the control binding agent are covalently coupled to the detection agent.

60. The test strip according to claim 48, wherein at least one of the first analyte binding agent and the control binding agent are non-covalently coupled to the detection agent.

61. The test strip according to claim 48, wherein the detection agent comprises particles.

62. The test strip according to claim 48, wherein the detection agent comprises colloidal gold.

63. The test strip according to claim 48, wherein the control agent is selected from the group consisting of an antibody, an engineered protein having binding sites non-specific for the analyte, a receptor that specifically binds to ligands other than the analyte, a ligand that specifically binds to receptors other than the analyte, an antigen, organic molecule, a hapten, IgG, immunoglobulins, albumins, casein, and globulin.

64. The test strip according to claim 48, wherein the control agent comprises rabbit IgG.

65. The test strip according to claim 48, wherein the control agent comprises rabbit anti-dinitrophenol IgG.

66. The test strip according to claim 48, wherein the control agent comprises dinitrophenol.

67. The test strip according to claim 48, wherein the one or more control measurement zones are positioned spatially apart from the analyte measurement zone on the test strip.

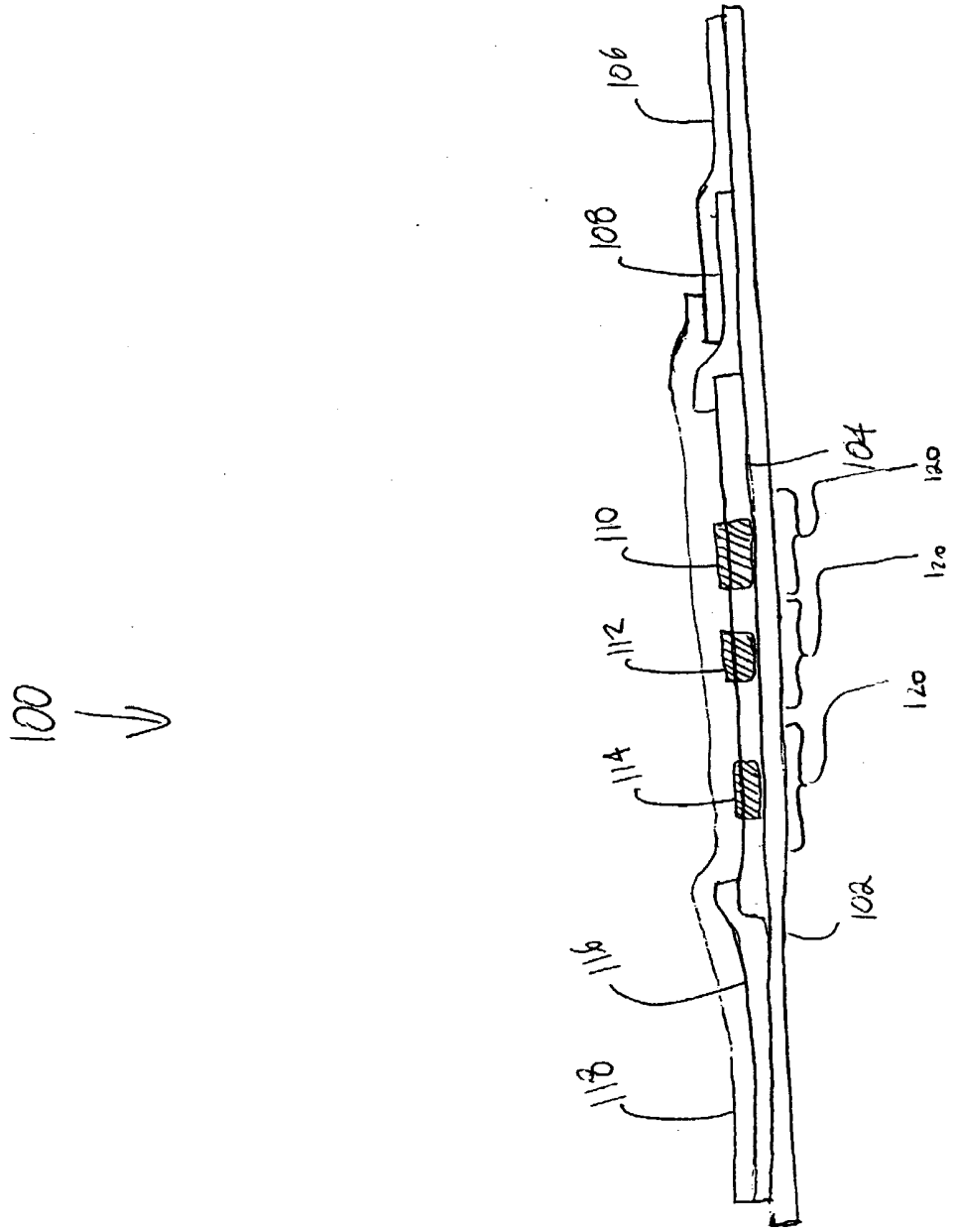

FIG. 1

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 99/27497

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/558 G01N33/543 G01N33/553

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 833 157 A (UNILEVER PLC ;UNILEVER NV (NL)) 1 April 1998 (1998-04-01) the whole document	1-67
A	WO 97 09620 A (AGEN BIOMEDICAL LTD ;RYLATT DENNIS BRIAN (AU); MOSS DEAN (AU); JAN) 13 March 1997 (1997-03-13) abstract claims 1-8 page 18	1-67
A	US 5 661 019 A (OH CHAN S ET AL) 26 August 1997 (1997-08-26) claims	1-67

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 April 2000	20/04/2000

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. ...nal Application No
PCT/US 99/27497

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